REMARKS

The specification is amended to correct certain typographical errors. In particular, the term "mg/d" is replaced by the term "mg/dl" on page 5, line 25. This is to be consistent with the correct designation of the concentration of lactate (in milligrams per deciliter) in the medium as taught at page 9, line 12 and in claim 3 as originally filed.

In addition, the term "mg/l" on page 9, line 11 is replaced by the term "mg/dl". This is to be consistent with correct designation of the concentration of glucose (in milligrams per deciliter) in the medium as taught at page 5, line 23 and in claims 1 and 3 as originally filed No new matter is added.

Claims 1-6 were pending in the application. As indicated above in the Listing of Claims, claims 1 and 3-9 will be pending and under active consideration upon entry of the present amendments. Claim 2 is cancelled herein, without prejudice and new claims 7-9 are added by amendment herein.

Rejection under Section 103

Claims 1-5 are rejected under Section 103 as obvious in view of a combination of U.S. Patent No. 5,663,051 (Ref. A), U.S. Patent No. 5,676,849 (Ref. B) and U.S. Patent No. 5,432,054 (Ref. C) taken with Guyton, 1991, Textbook of Medical Physiology, 8th ed., pp. 276-280; 330-331 and 752 (Ref. U). As stated in the Office Action, each of the cited patents is "relied upon for disclosure of methods for isolating nucleated fetal cells from maternal peripheral blood...wherein the methods encompass isolation of fetal nucleated red blood cells by density gradient centrifugation of maternal blood which has been modified by addition of various preparations."

With respect to Reference A, the Office Action asserts that the reference discloses a method of combining maternal blood "with a 'non-physiological', 'tissue' culture medium or 'PBS'".

With respect to Reference B, the Office Action asserts that it discloses the use of "generic 'non-physiological' conditions in order to modify the maternal blood and to include preferential lysis of maternal red blood cells."

With respect to Reference C, the Office Action asserts that the reference discloses the "use of 'non-physiological'" hypertonic conditions for modifying maternal blood and "suggests the use of 'PBS' solution as a 'non-physiological' hypertonic medium".

Reference U is asserted to demonstrate the normal physiological characteristics of blood.

Although the Office Action admits that the cited references "lack disclosure" with respect to particular non-physiological, characteristics or conditions" recited in the present claims, it asserts that the claimed invention is uncertain with regard to either starting, intermediate or final characteristics of maternal blood. In addition, although the Office Action admits that the specific pH and sodium concentration are outside the range of physiologically acceptable blood conditions, the Office Action concludes that the use of the present conditions would have been obvious given the teaching of References A, B and C with Reference U.

The Claimed Invention is Not Obvious

In reply, Applicant respectfully, but emphatically, traverses and submits that the presently claimed invention is not obvious in view of the combination of cited references. In order to more clearly explain the differences which patentably distinguish the present invention from the combination of teachings of the cited references, independent claim 1 (and all claims independent thereon) is amended herein to more precisely recite the specific steps of the claimed method and to clarify the starting materials, etc. and the specific, 'non-physiological' conditions which are critical to the presently claimed method. In addition, the language of claim 1 is clarified to avoid certain informalities associated with translation from a non-US priority application.

As made clear in the Summary of Invention, at page 3, line 26 through page 4, line 13, the present method comprises a first step step a: which involves:

> mixing maternal blood with a non-physiological tissue culture medium and an aqueous solution of citric acid, Na citrate and dextran.

Step a produces a "non-physiological tissue culture mixture" having specific characteristics of pH, osmolarity as well as glucose and lactate and ion concentrations in a fairly narrow range. As detailed on page 5, at lines 13-15 of the specification, when maternal blood is modified as in step a, the density of nucleated red blood cells (NRBCs) is changed relative to that of unmodified blood and the NRBCs become lighter than the other nucleated cells, such as lymphocytes and monocytes present in the original maternal blood sample.

Although, as explained in the specification at page 5, the reasons why this happens is not known. This is especially critical for the function of the present invention. In normal physiological conditions, the density distribution profile of NRBCs, including fetal NRBCs, overlaps with the density distribution profile of most other nucleated cells in maternal blood.

See, e.g., Sitar et al; 1997, Haematologica 82:5-10 (a copy of which is submitted herein as Reference C01 with the Information Disclosure Statement filed concurrently herewith).

In complete contrast, however, the present Applicant has unexpectedly found that, when maternal blood is in a non-physiological mixture having specific characteristics recited in claim 1, the density of the NRBCs becomes less than the other nucleated cells and the NRBCs can be separated from the rest of the nucleated cells present by discontinuous density gradient centrifugation. As clearly taught in the specification:

> The reason why cell densities of desired blood cells [NRBCs] change, rendering possible their separation according to the present invention is not clearly understood and represents a surprising feature of the present invention.

Page 5, lines 25-27. (emphasis added)

An additional step of the present method made possible by step a, comprises:

> introducing the mixture of step a into a separation device with a liquid having a density higher than maternal blood and containing an agent to aggregate red blood cells (RBCs).

The device is subjected to a single discontinuous density gradient centrifugation step. As explained at page 6, line 2, the lighter NRBCs "float at the interface with separating" liquid; whereas nucleated lymphocytes, monocytes, etc. sink to the bottom of the device. In addition, due to the action of the RBC aggregating agent, non-nucleated RBCs aggregate and fall to the bottom of the device. Finally, fetal NRBCs are identified among the isolated NRBCs by procedures known to those skilled in the art, including but not limited to such as anti-Ehb antibody or FISH, etc. See, the specification at page 6, lines 24-32; at page 10, lines 31 through page 11, line 4.

Nothing in the combination of references cited suggests, much less teaches, the presently claimed combination of steps to achieve isolation of fetal NRBCs from maternal blood.

The Office Action candidly admits that the disclosure of the combination of references does not teach or suggest the presently taught specific non-physiological conditions.

The Office Action asserts that one or more of the references teach phosphate buffered saline "PBS" as a non-physiological medium. This medium is completely different from the

non-physiological mixture employed in the presently claimed invention. Reference A, in particular, is alleged to suggest the use of PBS. Use of such a non-physiological medium is completely contrary to the method of the present invention. When blood is placed into PBS, the NRBCs and RBCs shrink. Water leaves the cells and the cells become more dense than under normal physiological conditions. Attention is directed to Bookchin et al., 1984, J. Lab. Clin. Med. 104:855-866, a copy of which is submitted as Reference C02 with the Information Disclosure Statement concurrently filed herewith. Thus, use of PBS, in contrast to the presently used non-physiological mixture, would make the NRBCs heavier rather than lighter. Thus, the NRBCs could not be separated from the other nucleated cells as presently claimed.

Moreover, it is noted that Reference A teaches a method using a pH of 7.4. References B and C teach methods using lysis of maternal RBCs.

None of the remaining teachings of any of the references adds anything else which would have suggested to one skilled in the art to employ the specifically claimed non-physiological conditions presently claimed. Many different combinations of conditions have been tried by the Applicant. The presently claimed combination, however, has been unique, in unexpectedly providing very advantageous conditions to allow isolation and identification of NRBCs in maternal blood. Such combination would not have been and was not obvious to one skilled in the art, absent the teaching of the present application.

Accordingly it is submitted this rejection is in error and must be withdrawn.

Claim 6 is rejected under Section 103 as obvious in view of Reference A, B and C taken with Reference U as applied to claims 1-5 and further in view of U.S. Patent No. 4,424,132 (Ref. D), GB-2-75376 (Ref. N), and FR 7708053 (Ref. O). The additional references are relied upon as allegedly showing a large variety of cell separation devices which are suitable for use in the claimed method.

Applicant respectfully, but emphatically disagrees. Essentially, for all the reasons recited above with respect to the rejection of claims 1-5, Applicant submits that this rejection is in error. The combination of References A, B, C and U does not suggest the presently claimed method for isolating NRBCs using the specifically detailed method comprising mixing maternal blood with tissue culture medium, and an aqueous solution of citric acid, Na citrate and dextran to form a mixture with the specific narrowly recited ranges of pH, osmolarity and concentrations of glucose, lactate and ions so that the NRBCs become lighter and other non-RBCs in maternal blood become more dense and subjecting such mixture to

discontinuous density gradient centrifugation in the presence of an RBC aggregating agent so that the RBCs fall to the bottom and NRBCs can be isolated in a single centrifugation step.

Nothing in the disclosure of each and every one of the additional references, taken together with each other and with References A, B, C and U suggest, much less teach, either the specific method, the specific device of claim 6 or the combination of same. Thus, this rejection is in error and must be withdrawn.

Rejection under Section 112, 2d Para

Claims 1-6 are rejected under Section 112, 2d paragraph as allegedly indefinite for a number of reasons.

In particular, claims 1 and 3 are alleged to be indefinite because it is "unclear whether the claimed characteristics including pH, osmolarity, concentration of various ions, glucose and lactate are the characteristics of a 'non-physiological culture medium' when combined with an "aqueous solution before addition to a maternal blood or whether the claimed characteristics are the modified maternal blood characteristics after addition of a 'non-physiological culture medium' and an aqueous solution." These claims are also stated to be indefinite regarding the concentrations of glucose and lactate and with respect to the terms "mg/d" and "mg/dl". Further, the claims are alleged to be indefinite with respect to the terms "RBCs" and "NRBCs". Claim 1 is alleged indefinite with respect to the phrase "appropriate procedures". Claim 6 is alleged to be indefinite with respect to the use of "numbers" "(1)", etc.

In response, claim 1, (and all claims dependent thereon) are amended herein to recite more clearly and precisely the present invention.

In particular, claim 1, as amended, clearly indicates that the pH, osmolarity, etc. parameter, recited in step (a) are the characteristics of the "non-physiological tissue culture mixture" formed by mixing:

1) maternal blood

2) tissue culture medium; and

3) an aqueous solution containing citric acid, Na citrate and dextran.

Attention is directed to the teaching of the specification in the Description of the Invention at page 5, line 10 through page 6, line 11 and to the Example at page 7, line 6 through page 11, line 4. As explained in the teaching of the specification at pages 5-6 (and as illustrated in the working Example at pages 7-11), the method of the invention entails: transferring maternal blood into non-physiological culture medium in which the nucleated

red blood cells (NRBCs) have a lower than normal density and in which lymphocytes and monocytes have a higher than normal density. The characteristics of the non-physiological medium which is a mixture containing the maternal blood as provided at page 5, lines 15-24. There are the same characteristics as recited in claim 1.

Attention is further directed to the illustrative Example at pages 7-11. As stated therein, beginning at line 7, a sample of maternal blood, is transferred to tissue culture medium having a composition recited in Table 2 at page 8. The tissue culture medium specified in Table 2 is a "non-physiological medium". As clearly stated at page 9, lines 2-4: a specified amount of "an aqueous solution are added", said solution containing citric acid, Na citrate and dextran, "thus obtaining the following non-physiological conditions". The conditions specified are thus the specific non-physiological conditions of the mixture of maternal blood, tissue culture medium and aqueous solution, as recited in dependent claim 3.

Claims 1 and 3 are further amended to correct the typographical errors with respect to the concentrations of glucose and lactate with respect to the abbreviated terms for "milligrams/deciliter".

Claims 1, as amended, also clearly recites that the abbreviation "NRBCs" is intended to mean "nucleated red blood cells" and that the abbreviation "RBC" is intended to mean "red blood cells". These terms are used consistently in the amended claims. These terms are standard in the art and would be appropriately understood by those skilled in the art.

Claim 6, as amended, avoids the use of the "numbers" for various parts of the device used in this claim. As indicated by the Office Action, such numbers are not necessary.

Finally, new claims 7-8 are added to recite methods, respectively, for identifying and for identifying and counting fetal nucleated red blood cells (NRBCs), using the method of the invention comprising mixing a maternal blood sample with tissue culture medium and an aqueous solution containing citric acid, Na citrate and dextran to form a non-physiological tissue culture mixture having the characteristics as recited in claim 1, transferring the mixture to a separation device having a liquid with a density higher than the maternal blood and containing a RBC aggregating agent, isolating the NRBCs and identifying the NRBCs or identifying and counting the NRBCs. Claims 7 and 8 avoid any indefiniteness of the original claims and are fully supported by the specification as filed.

New claim 9 recites the method of claim 7 in which the separation device is as illustrated in Fig. 1. Claim 9 is fully supported by the specification as filed.

In light of the above amendments, it is respectfully submitted that the rejection based on Section 112 is avoided and must be withdrawn.

CONCLUSION

In light of the above amendments and remarks, the Applicant respectfully requests that the Examiner reconsider this application with a view towards allowance. The Examiner is invited to call the undersigned attorney if a telephone call could help resolve any remaining items.

Respectfully submitted,

Date: September 17, 2003

Geraldine F. Baldwin (Reg. No. 31,232)
PENNIE & EDMONDS LLP
1155 Avenue of the Americas
New York, New York 10036-2711
(212) 790-9090